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EFFECTS OF N^G-NITRO-L-ARGININE AND L-ARGININE ON REGIONAL CEREBRAL BLOOD FLOW IN THE CAT

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SUMMARY

1. We studied the effects of N^G-nitro-L-arginine (NOLA), a potent inhibitor of the L-arginine–nitric oxide pathway, and L-arginine, the precursor of nitric oxide, on regional cerebral blood flow, electrocortical activity and *ex vivo* cerebrovascular reactivity in the cat. Flow was measured via radiolabelled microspheres, and vascular responses were studied by measuring isometric tension of isolated middle cerebral arterial rings.

2. NOLA (30 mg kg^{-1} bolus followed by 1 mg kg^{-1} min^{-1} infusion) caused an approximately 40 mmHg elevation in the mean arterial blood pressure, a regionally heterogenous increase of the regional cerebrovascular resistance and a decrease in the regional cerebral blood flow 15 and 40 min after the start of its administration. In contrast L-arginine (30 mg kg^{-1} bolus followed by 10 mg kg^{-1} min^{-1} infusion) did not alter blood pressure, cerebrovascular resistance nor regional cerebral blood flow 15 min after the start of its administration. The NOLA-induced changes in tissue flow were the most pronounced in the cerebellum, pituitary and medulla oblongata, whereas there was no decrease in the flow of the cortex and white matter.

3. NOLA caused characteristic changes in total fronto-occipital EEG power and in power spectra which were unlikely to have been due to cerebral ischaemia. In addition, the *ex vivo* reactivity of the middle cerebral arteries showed signs of impaired endothelial nitric oxide synthesis: there were enhanced noradrenaline-induced contractions and *N*-ethoxycarbonyl-3-morpholino-sydnonimine (SIN-1)-induced relaxations and markedly attenuated acetylcholine- and ATP-induced relaxations after NOLA treatment.

4. The present data indicate that resting cerebral blood flow and cerebrovascular resistance are regulated by nitric oxide derived from L-arginine in a regionally heterogenous way and that exogenous L-arginine availability is not a limiting factor in this nitric oxide generation. Possibly, both the vascular endothelium and the neurons contribute to this basal nitric oxide release.

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INTRODUCTION

Nitric oxide (NO), produced from the terminal guanidino group of L-arginine, is an important biological mediator which accounts for the vasorelaxant activity of endothelium-derived relaxing factor (EDRF) (Sakuma, Stuehr, Gross, Nathan & Levi, 1988; Moncada, Palmer & Higgs, 1989). Guanidino-monosubstituted derivatives, like N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine (NOLA), are potent inhibitors of the L-arginine–NO pathway (e.g. Moncada *et al.* 1989; Gross, Stuehr, Aisaka, Jaffe, Levi & Griffith, 1990; Moore, al-Swayeh, Chong, Evans & Gibson, 1990).

Recent studies have shown that NO is also synthesized in a variety of cells including macrophages and neurons (Bredt & Snyder, 1989; Knowles, Palacios, Palmer & Moncada, 1989, 1990; Moncada *et al.* 1989; Schmidt, Wilke, Evers & Böhme, 1989; Bredt, Hwang & Snyder, 1990; Garthwaite, 1991). NO is involved in the regulation of blood pressure and in the physiological inhibition of platelet adhesion and aggregation (Aisaka, Gross, Griffith & Levi, 1989; Moncada *et al.* 1989; Mustafa, Mester, Thiemermann, Hecker & Vane, 1990). Recent studies also suggested that a basal release of NO is responsible for the maintainance of vascular resistance and normal blood flow in the heart, brain and kidney (Bhardwaj & Moore, 1989; Busija, Leffler & Wagerle, 1990; Faraci, 1990; Gardiner, Compton, Bennett, Palmer & Moncada, 1990*a*, *b*; Rosenblum, Nishimura & Nelson, 1990; Humphreys, Carr, Nicol, Tomlinson & Connor, 1991).

NO has also been suggested to be an important transmitter in the central nervous system which is released basally and upon the activation of *N*-methyl-D-aspartate and glutamate receptors (Bredt & Snyder, 1989; Knowles *et al.* 1989, 1990; Schmidt *et al.* 1989; Bredt *et al.* 1990; Garthwaite, 1991). The involvement of NO in the mediation of coupling between the neural activity of the brain and cerebral blood flow has also been proposed (Gally, Montague, Reeke & Edelman, 1990). There is a regionally heterogenous distribution of NO synthase in the central nervous system (Bredt *et al.* 1990). Various regions of the brain produce different amounts of NO (Förstermann, Gorsky, Pollock, Schmidt, Heller & Murad, 1990).

In the present study using NOLA, a specific inhibition of NO synthase (Moncada *et al.* 1989; Gross *et al.* 1990; Moore *et al.* 1990), and L-arginine, the precursor of NO (Sakuma *et al.* 1988; Moncada *et al.* 1989), we further investigated the role of the L-arginine–NO pathway in the brain. We studied whether *in vivo* blockade of NO synthase or application of L-arginine alters regional cerebral blood flow and vascular resistance and whether there is any regionality in the NO-mediated regulation of cerebral circulation in the anaesthetized cat. Whether these treatments influence the electrical activity of the cerebral cortex or *ex vivo* cerebrovascular reactivity was also studied.

METHODS

Surgical procedures

The experiments were carried out in nineteen male cats, weighing 2·5–3·5 kg, anaesthetized with a combination of 50 mg kg^{-1} chloralose and 200 mg kg^{-1} urethane given intraperitoneally. The trachea, both femoral arteries (the right one for blood pressure measurement and the left one for withdrawal of blood samples), the left brachial artery (for withdrawal of blood samples) and the right femoral vein (for administration of drugs) were cannulated. After an intercostal thoracotomy,

a small slit was made in the tip of the left atrium and a small polyethylene catheter was inserted. A purse string suture was used to secure the catheter in place. Heparin was given in a dose of 250 U kg^{-1} I.V. to prevent clotting. The head of the animal was mounted in a stereotaxic head-holder. The cats were artificially ventilated (Harvard Respirator, Southnatick, MA, USA). The volume and rate of respiration were set to maintain arterial P_{O_2} and P_{CO_2} at approximately 120 and 36–39 mmHg, respectively. Arterial pH was kept constant between 7·3 and 7·35.

Routinely recorded parameters

Systemic arterial pressure (Statham p23/d electromanometer, Spectamed, Hato Rely, Puerto Rico) and end-tidal CO_2 (End-tid IL 200 CO_2 monitor, Instrumentation Laboratory, Lexington, MA, USA) were continuously recorded on a Grass polygraph (Model 7D, Quincy, MA, USA). Rectal temperature was maintained at 37 °C by an infra-red lamp and a Yellowspring temperature regulator (Model 73/A, Yellow Spring, OH, USA). Arterial blood gas values (P_{O_2}, P_{CO_2}, pH) were measured in femoral arterial blood samples by a Radiometer Blood Gas Analyzer (ABL-30, Copenhagen, Denmark). Arterial glucose concentrations were determined by a Beckman Glucose Analyzer (Fullerton, CA, USA).

Cerebral blood flow measurement

The measurement of regional cerebral blood flow (rCBF) was performed with radiolabelled microspheres (15 μm in diameter) (Heymann, Payne, Hoffmann & Rudolph, 1977; Tanaka, Greenberg, Gonatas & Reivich, 1985; Mayhan, Amundsen, Faraci & Heistad, 1988). Two or three out of five microspheres (^{57}Co, ^{113}Sn, ^{85}Sr, ^{95}Nb and ^{46}Sc) (Du Pont, Boston, MA, USA) were used to measure cerebral blood flow in each animal. Approximately $3·5 \times 10^6$ spheres were injected for each measurement into the left atrium over a 10 s period followed by a 30 s flush of 2 ml of saline. Two reference blood samples were withdrawn from the left femoral and brachial arteries using a Harvard pump (Dover, MA, USA) set at 1 ml min^{-1} beginning 20 s before the injection and continuing for 80 s after the flush. An average count of these two samples was then calculated and used as a reference. At the end of the experiment the animal was killed by rapid exsanguination and the brain and the spinal cord were removed.

Tissue samples weighing approximately 100 mg were prepared from the following areas of the brain: parietal cortex, thalamus, hypothalamus, pituitary, cerebellar cortex, white matter (corpus callosum) and medulla oblongata. Similarly, samples from the cervical, thoracic and lumbar spinal cord were prepared. All tissue samples were weighed and placed in 7 ml borosilicate glass scintillation vials (Kimble, Toledo, OH, USA) and counted in a Packard multichannel autogamma scintillation spectrometer (5000 Series, Downers Grove, IL, USA) with a 3 in through-hole NaTl crystal. The energy levels of the window settings for the five isotopes were as follows: ^{57}Co, 80–150; ^{113}Sn, 340–430; ^{85}Sr, 460–560; ^{95}Nb, 700–800; and ^{46}Sc, 810–1400 keV. The overlap of activity among isotopes was subtracted to obtain corrected counts for each isotope by solving simultaneous equations using overlap coefficients from pure isotope standards. Blood flow was calculated from the equation

$$\text{rCBF} = (\text{RW}) \times (\text{TA}/\text{BA}),$$

where RW is the pump withdrawal rate (in ml min^{-1}), BA is the blood activity and TA is the tissue sample activity.

Using this methodology, the microspheres are distributed in proportion to the distribution of cardiac output, and each tissue section contains microspheres to make the measurements reliable (see also Heymann *et al.* 1977; Tanaka *et al.* 1985).

Regional vascular resistances (VR) were calculated as blood pressure/regional blood flow.

EEG analysis

Fronto-occipital EEG was recorded on paper via a Grass polygraph (Model 7D, Quincy, MA, USA) and on audio-cassette via a multichannel EEG recorder (Model C4, A. R. Vetter Co., Rebersburg, PA, USA) connected to a Sony tape-recorder (TC-K 700 ES). The EEG was analysed using a Compaq Deskpro 286 PC (Houston, TX, USA) and the Rhythm software (Version 7·0, Stellate Systems, Quebec, Canada). Using this program, we sampled consecutive 20 s epochs, with a sampling rate of 128 points s^{-1}. Samples from the EEG activity were obtained 3 min before the start of application of NOLA or L-arginine (control) as well as 5, 10, 15, 30 and 60 min after the start of the infusion. Power spectra were computed in the following frequency bands: δ, 0·75–3·75 Hz; θ, 4–7·75 Hz; α, 8–12·75 Hz; β, 13–20·25 Hz; β_1, 20·5–25·25 Hz and β_2, 25·5–31 Hz.

Total EEG power at different time points during the experiment is expressed as percentage of the initial power. Band power values are expressed as a percentage of the total power of the spectrum at each time point.

Isometrical tension recording of isolated blood vessels

Two millimetre-long segments of the middle cerebral artery were placed on two L-shaped stainless-steel specimen holders (0·1 mm in diameter), one of which was attached to a Grass FT03 force transducer (Quincy, MA, USA). The position of the other holder could be adjusted by a micromanipulator. Isometric tension of the vessels was recorded on a Grass polygraph (Model 7D, Quincy, MA, USA). The preparations were immersed into a tissue chamber containing Krebs–Henseleit solution of the following composition (in mM): NaCl, 119; KCl, 4·6; $CaCl_2$, 1·5; $MgCl_2$, 1·2; $NaHCO_3$, 15; NaH_2PO_4, 1·2; glucose, 6; and aerated with a gas mixture containing 95% O_2 and 5% CO_2. The temperature of the solution was 37 °C and the pH was 7·4 (Högestatt, Andersson & Edvinsson, 1983). The vessels were first incubated for 45 min at a tension of 500 mg. After this, the effects of 127 mM-KCl solution, noradrenaline, acetylcholine, ATP and *N*-ethoxycarbonyl-3-morpholino-sydnonimine (SIN-1) were tested. The constrictor agents were applied on the resting tone, whereas dilator agents were applied after a stable precontractile tone had been induced by 5×10^{-6} M-prostaglandin F_2. All measurements were carried out in the presence of 5×10^{-6} M-indomethacin and 5×10^{-7} M-propranolol to block the production of metabolites of the enzyme cyclo-oxygenase and β-receptor activation, respectively. Contractile responses were expressed in milligrams and dilator responses were expressed as the percentage of the initial tension caused by prostaglandin F_2.

Experimental protocols

After completion of the surgical procedures, a stabilization period of 30 min was allowed. The animals were divided then into three groups. The animals in the first group (five cats) were rapidly exsanguinated and the responses of the cerebral vessels were studied *in vitro*. In the second and third group (eight and six cats, respectively) the effect of N^G-nitro-L-arginine (NOLA) or L-arginine infusion was studied. NOLA infusion consisted of a 30 mg kg^{-1} initial bolus in 5 ml saline, followed by a 1 mg kg^{-1} min^{-1} continuous infusion at a rate of 0·2 ml min^{-1} and the L-arginine infusion was a 30 mg kg^{-1} initial bolus in 5 ml saline, followed by a 10 mg kg^{-1} min^{-1} continuous infusion at the same rate using a Harvard infusion/withdrawal pump (Dover, MA, USA).

First, regional cerebral blood flow was measured under control conditions in each animal. After 5 min, the infusion of NOLA or L-arginine was started and maintained for 60 min. The second ($n = 8$) and third ($n = 6$) CBF measurements were performed 15 and 40 min after the start of the infusion in the NOLA-treated animals. The second CBF measurement was performed 15 min after the start of the infusion in the L-arginine-treated animals ($n = 6$). The cats were killed 60 min after the start of the infusion by rapid exsanguination.

Drugs

The following drugs were used: acetylcholine, adenosine triphosphate (ATP), L-arginine, indomethacin, noradrenaline, propranolol, prostaglandin F_2, N^G-nitro-L-arginine (all from Sigma Chemical Co., St Louis, MO, USA) and SIN-1 (a generous gift from Casella AG, Frankfurt, Germany). Indomethacin and prostaglandin F_2 were dissolved in 50% ethanol; all other drugs were dissolved in 0·9% NaCl solution. N^G-Nitro-L-arginine was sonicated before use. All concentrations are expressed as final molar organ chamber concentrations.

Data analysis

All values in the text and figures are presented as mean ± standard error of the mean; for the *in vivo* measurements the number of animals studied and for the organ chamber studies the number of rings tested were expressed by n. All vascular responses studied have been performed in at least one (usually two to four) vessel segment from each animal. If two groups were compared, Student's paired and unpaired t tests were applied, as appropriate. If responses were compared between more than two groups, data were analysed by one-way analysis of variance to assess overall significance of group differences. If a statistically significant F value was observed, an appropriate multiple comparison method (Dunnett's test) was applied to evaluate the difference between any two groups.

RESULTS

Physiological variables

Table 1 demonstrates physiological variables at each time point when rCBF was measured. There were no significant differences in the initial parameters between the two groups of cats prior to NOLA or L-arginine administration. After administration of NOLA, a significant increase in the mean arterial pressure was observed. The onset of action occurred within the first 10 min of administration, and did not change over the 60 min period of the experiment (Fig. 1). No changes in the physiological parameters were observed after the application of L-arginine.

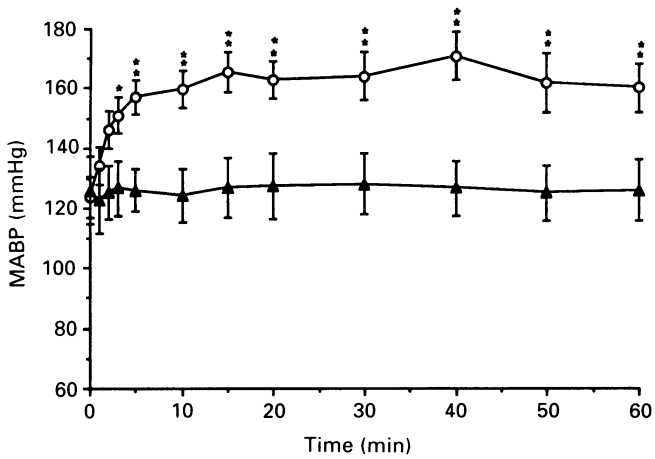

Fig. 1. Time course of the effects of I.V. administration of NOLA (○, $n = 8$) and L-arginine (▲, $n = 6$) on mean arterial blood pressure (MAP, mmHg). Values are mean ± S.E.M. *, ** indicate significant differences from the control level ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively).

TABLE 1. Physiological conditions at time of rCBF determination before and after NOLA or L-arginine administration

		After NOLA			
	Before NOLA	15 min	40 min	Before L-arginine	15 min after L-arginine
P_{a,CO_2} (mmHg)	39·6 ± 3·2	35·7 ± 2·5 n.s.	39·3 ± 2·6 n.s.	40·9 ± 3·4	40·3 ± 5·6 n.s.
P_{a,O_2} (mmHg)	123 ± 4	118 ± 6 n.s.	115 ± 10 n.s.	125 ± 4	124 ± 7 n.s.
pH_a	7·32 ± 0·01	7·33 ± 0·01 n.s.	7·33 ± 0·02 n.s.	7·32 ± 0·02	7·32 ± 0·02 n.s.
Glucose (mg ml^{-1})	177 ± 16	180 ± 15 n.s.	215 ± 19 n.s.	157 ± 29	175 ± 20 n.s.
MAP (mmHg)	123 ± 7	166 ± 7 **	171 ± 8 **	126 ± 11	127 ± 11 n.s.
HR (beats min^{-1})	227 ± 16	201 ± 20 n.s.	197 ± 34 n.s.	200 ± 10	196 ± 11 n.s.

** Significant difference from control, $P < 0{\cdot}01$. Not significant, n.s.

Regional cerebral blood flow

Control regional blood flow values were not different in the two groups of cats prior to NOLA or L-arginine administration. NOLA infusion caused a significant decrease in the rCBF 15 min after the start of its administration in the thalamus, cerebellar

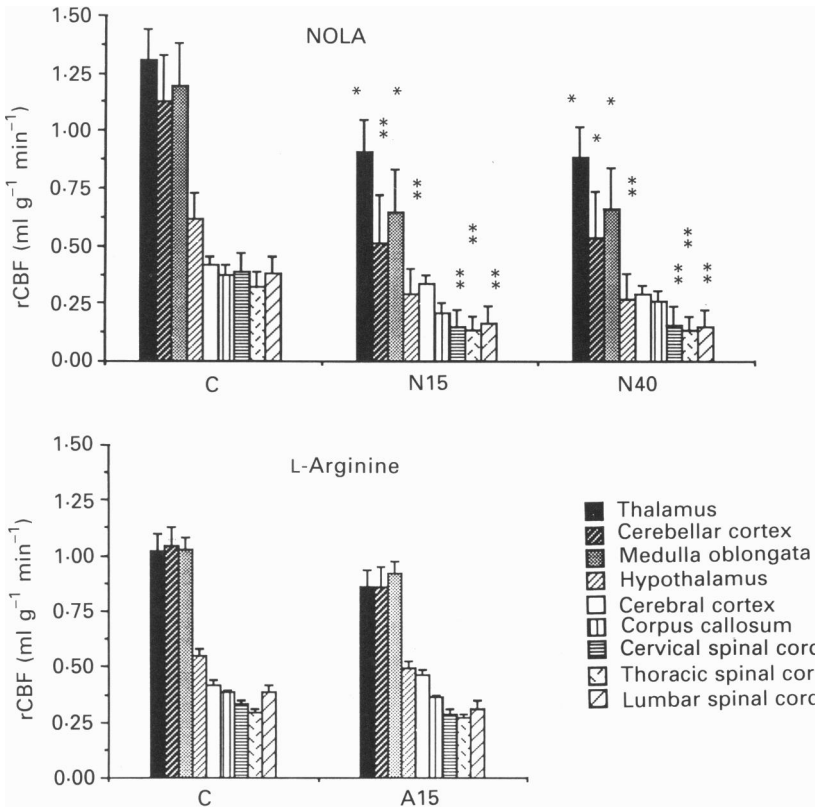

Fig. 2. Effect of NOLA (upper panel) and L-arginine (lower panel) on regional cerebral blood flow (rCBF, ml g^{-1} min^{-1}) in the thalamus, cerebellar cortex, medulla oblongata, hypothalamus, cerebral cortex, corpus callosum, and cervical, thoracic and lumbar spinal cord. C, control rCBF; N15 and N40, rCBF 15 and 40 min after the start of NOLA administration ($n = 8$ and 6, respectively). Lower panel: C, control; A15, rCBF 15 min after the start of L-arginine administration ($n = 6$). Values are mean ± S.E.M. *, ** indicate significant differences from the control level ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively).

cortex, medulla oblongata, hypothalamus, cervical, thoracic and lumbar spinal cord (Fig. 2) and pituitary (Fig. 3). These decreases were still present 40 min after the start of infusion of NOLA (Figs 2 and 3). In contrast, cortical blood flow and the flow of the corpus callosum did not show significant depression either 15 or 40 min after the start of I.V. NOLA administration (Fig. 2). NOLA caused maximal decrease in the blood flow of the pituitary (to approximately 33% of the initial flow), cerebellum, hypothalamus, spinal cord and medulla oblongata (to approximately

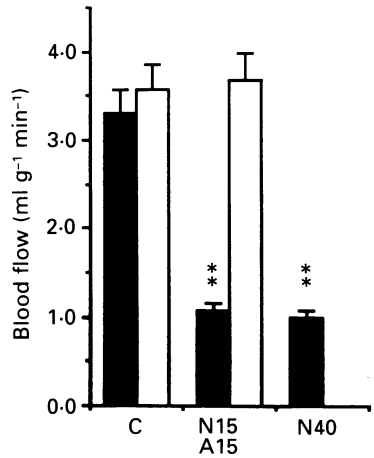

Fig. 3. Effect of NOLA (▨) and L-arginine (□) on the blood flow of the pituitary (blood flow, ml g^{-1} min^{-1}). C, control; N15 and N40, blood flow 15 and 40 min after the start of NOLA administration ($n = 8$ and 6, respectively); A15, blood flow 15 min after the start of L-arginine administration ($n = 6$). Values are mean ± S.E.M. ** indicate significant differences from the control level ($P < 0{\cdot}01$).

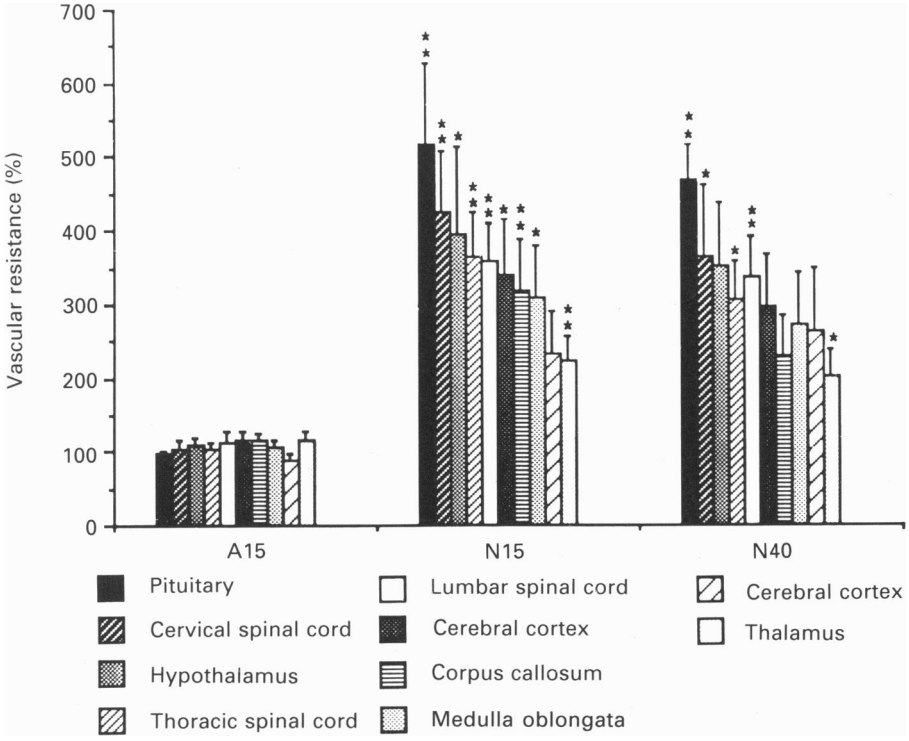

Fig. 4. Effect of NOLA and L-arginine on regional cerebrovascular resistance. Control vascular resistance was set to 100%. N15 and N40, vascular resistance 15 and 40 min after the start of NOLA administration ($n = 8$ and 6, respectively); A15, vascular resistance 15 min after the start of L-arginine administration ($n = 6$). Values are mean ± S.E.M. *, ** indicate significant differences from the control level ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively).

50–55% in each region). L-Arginine infusion did not alter regional cerebral and pituitary blood flow after 15 min (Figs 2 and 3).

Regional cerebrovascular resistance

In addition to marked decreases in regional blood flow, marked increases in the regional vascular resistance (VR) were observed 15 and 40 min after the

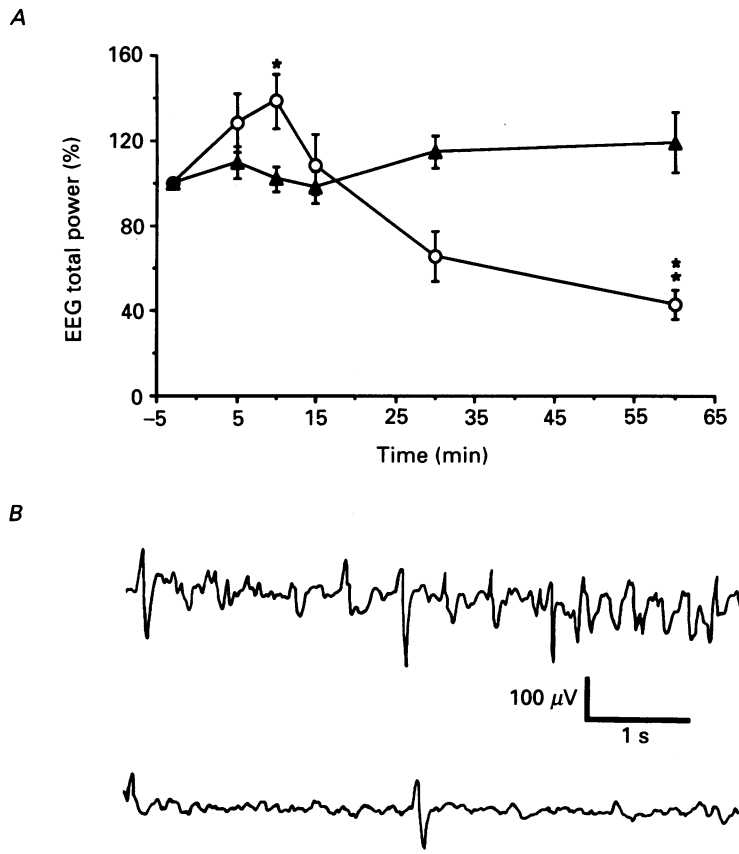

Fig. 5. *A*, effect of NOLA (○) and L-arginine (▲) on total EEG power. NOLA and L-arginine were administered at 0 time point ($n = 8$ and $n = 6$ respectively). EEG power is expressed as a percentage of the initial power. These initial values did not differ from each other significantly. Values are mean ± S.E.M. *, ** indicate significant differences from the initial control level ($P < 0.05$ and $P < 0.01$, respectively). *B*, original EEG tracings showing the effect of NOLA on electrocortical activity. Upper panel, EEG before application of NOLA; lower panel, EEG 60 min after NOLA infusion.

administration of NOLA. This change was the most pronounced in the pituitary, spinal cord, hypothalamus and cerebellum (approximately 3- to 5-fold increases) (Fig. 4). In contrast to the effect of NOLA, L-arginine infusion did not alter regional VR after 15 min.

Electrocortical activity

NOLA caused a biphasic change in the EEG power. There was a significant enhancement of power after the start of NOLA infusion, and there was then a gradual decrease (Fig. 5). Both the enhancement and the depression of the EEG power at 10 and 60 min were accompanied by changes in the power spectra (Table 2). In contrast, L-arginine infusion did not alter total EEG power (Fig. 5) or power spectra (data not shown).

TABLE 2. EEG power spectra before and 10 and 60 min after NOLA administration

	Control	10 min	60 min
δ	34·5±2·7	39·6±3·6	31·4±4·2
		n.s.	n.s.
θ	38·6±1·5	33·4±1·3	36·9±1·2
		*	n.s.
α	17·4±1·6	16·8±1·7	18·2±2·3
		n.s.	n.s.
β	7·8±0·8	8·1±0·9	9·5±1·2
		n.s.	n.s.
β_1	1·4±0·2	1·7±0·3	3·1±0·5
		n.s.	*
β_2	0·6±0·1	0·6±0·1	1·0±0·1
		n.s.	*

* Significant difference from control, $P < 0{\cdot}05$.

Ex vivo cerebrovascular reactivity

No significant differences were found between the contractile responses to 127 mM-K^+ in the different groups of animals; the absolute values of these contractile responses were 941±55 mg (control vessels, $n = 26$), 944±67 mg (vessels from L-arginine-treated cats, $n = 15$) and 874±33 mg (vessels from NOLA-treated cats, $n = 25$).

There was a significant enhancement in the noradrenaline-induced contractile response and the SIN-1-induced relaxant response after *in vivo* NOLA treatment, whereas there was a significant depression of the acetylcholine- and ATP-induced relaxations. In contrast, *in vivo* L-arginine treatment did not alter these vascular responses (Fig. 6).

DISCUSSION

Blood pressure and heart rate

Recent studies reported marked increases in blood pressure in rats and guinea-pigs after I.V. administration of L-NMMA or NOLA (Aisaka *et al.* 1989; Rees, Pamer & Moncada, 1989; Gardiner *et al.* 1990*a*, *b*; Gross *et al.* 1990; Mustafa *et al.* 1990; Wang & Pang, 1990; Humphreys *et al.* 1991). The present results on cats confirm these data and support the suggestion that a basal release of nitric oxide plays an important role in the blood pressure maintenance. There was no significant alteration in the heart

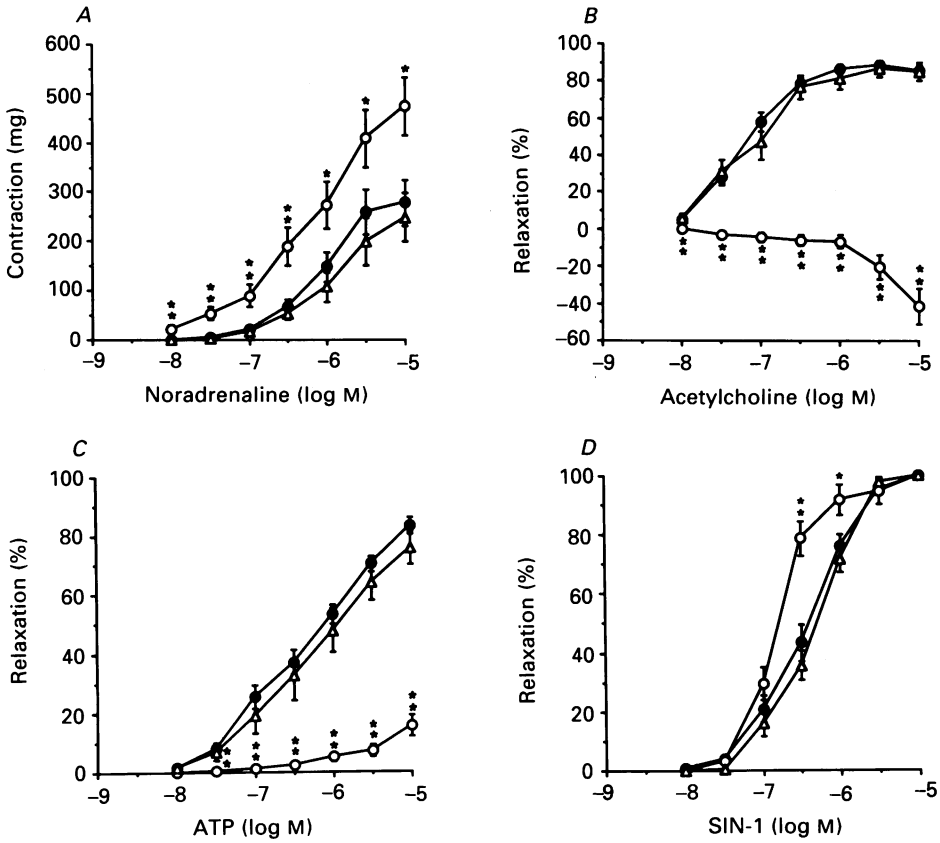

Fig. 6. *A*, concentration–response curves to noradrenaline in the middle cerebral arteries of control animals (●, $n = 22$), of animals after NOLA infusion (○, $n = 16$) and of animals after L-arginine infusion (△, $n = 15$), *B*, concentration–response curves to acetylcholine in the middle cerebral arteries of control animals (●, $n = 26$), of animals after NOLA infusion (○, $n = 25$) and of animals after L-arginine infusion (△, $n = 15$). *C*, concentration–response curves to ATP in the middle cerebral arteries of control animals (●, $n = 25$), of animals after NOLA infusion (○, $n = 25$) and of animals after L-arginine infusion (△, $n = 15$). *D*, concentration–response curves to SIN-1 in the middle cerebral arteries of control animals (●, $n = 22$), of animals after NOLA infusion (○, $n = 23$) and of animals after L-arginine infusion (△, $n = 15$). The vessels were precontracted with prostaglandin F_2 (5×10^{-6} M; *B*, *C* and *D*). Values are mean ± S.E.M. *, ** indicate significant differences from control response ($P < 0.05$ and $P < 0.01$, respectively).

rate either after NOLA or after L-arginine administration, as was described for NOLA in rats (Wang & Pang, 1990) and rabbits (Humphreys *et al.* 1991). In contrast to NOLA, L-NMMA causes a decrease in the heart rate (Aisaka *et al.* 1989; Rees *et al.* 1989; Gardiner *et al.* 1990*a*, *b*). The cause of this difference between the effects of these two arginine analogues on heart rate is not yet known.

Regional cerebral blood flow and cerebrovascular resistance

NOLA infusion caused a marked and regionally heterogenous decrease in CBF and an increase in vascular resistance. Recent observations have shown that L-NMMA

and NOLA cause an endothelium-dependent constriction of blood vessels *in vitro* (Gold, Wood, Byrns, Fukuto & Ignarro, 1990; Moore *et al.* 1990) and decrease the diameter of the internal carotid (Gardiner *et al.* 1990*a*), basilar (Faraci, 1990) and pial (Busija *et al.* 1990; Rosenblum *et al.* 1990) blood vessel *in vivo*. NOLA has also been shown to induce an increase in the total vascular resistance of the brain in the conscious rabbit (Humphreys *et al.* 1991). The present study suggests that the NOLA-induced constriction of the cerebral vessels is able to decrease tissue blood flow in the brain, providing evidence that basal NO is involved in the maintenance of cerebral perfusion. This basal release of nitric oxide contributes differentially to the maintenance of normal flow in different cerebral regions. The present study suggests that there is a pronounced NO-mediated regulation of the resting perfusion, e.g. in the pituitary, cerebellum and hypothalamus, whereas NO is not (or less) involved in the maintenance of flow in cortex and white matter. The physiological importance of this marked heterogeneity is not yet known.

Recent studies have compared the distribution of NO synthase and that of NO-synthesizing activity in the rat brain (Bredt *et al.* 1990; Förstermann *et al.* 1990). These studies showed that the cerebellum, the hypothalamus and the pituitary have a large amount of NO synthase/NO releasing activity, whereas the cerebral cortex is a region which has less NO synthase and produces less NO. It is interesting that in our studies, performed in the cat, blood flow into cerebellum, hypothalamus and pituitary was found to show marked decreases after NOLA, whereas there was no change in the blood flow in the cortex and white matter. These similarities raise the possibility that NO, responsible for the maintenance of regional blood flow and vascular resistance in the brain, originates (in part) from the neural tissue. Our EEG findings (see below), as well as several recent studies showing that NO is involved in the regulation of central nervous system functions (see Groll-Knapp, Haider, Kienzl, Handler & Trimmel, 1988; Moore, Oluyomi, Babbedge, Wallace & Hart, 1991), also support a direct neural action of NOLA. In contrast, the *ex vivo* responses of the middle cerebral arteries prepared from the NOLA-treated animals (see below) prove that *in vivo* NOLA treatment is able to inhibit the NO release from the cerebrovascular endothelium in large vessels (and probably also in the microcirculation), suggesting the presence of an endothelial component in the present circulatory changes. To further clarify the source of NO responsible for the haemodynamic changes in the present experiments, additional studies are needed, e.g. with various analogues of L-arginine with differential potency for crossing the blood–brain barrier.

EEG changes

Marked changes were found after NOLA administration in the total fronto-occipital EEG as well as in power spectra. An indirect effect of NOLA (causing these changes through cortical ischaemia) seems to be unlikely, considering the fact that NOLA did not alter cortical blood flow. Furthermore, the observed changes in EEG spectra (early decrease in θ-activity and late increase in β_1- and β_2-activity) are not characteristic of cerebral ischaemia (see Mabe, Nagai, Tagaki, Umemura & Ohno, 1986; Daly & Pedley, 1990; Scavini, Rozza, Bo, Lanza, Favalli, Savoldi & Racagni, 1990).

Cerebrovascular reactivity

Marked alterations were found when the reactivity of vessels of the NOLA-treated animals was studied. The enhancement of the adrenergic contractions, and the attenuation of the purinergic and cholinergic relaxations in the vessels of the NOLA-treated animals, are consistent with the view that these agents release NO (see also e.g. Rees *et al.* 1989; Busija *et al.* 1990; Faraci, 1990; Gold *et al.* 1990; Moore *et al.* 1990; Mülsch & Busse, 1990; Mustafa *et al.* 1990; Rosenblum *et al.* 1990). Similar changes in responsiveness were observed after *in vitro* blockade of NO synthase by NOLA (10^{-4} M) in control middle cerebral arterial segments (authors' unpublished observations). Our finding that NOLA treatment enhanced SIN-1-induced (i.e. endothelium-independent, NO-mediated; Feelisch & Noack, 1987) relaxations has also been observed recently after *in vivo* or *in vitro* treatment with L-NMMA or NOLA (Flavahan & Vanhoutte, 1989; Moncada, Rees, Schultz & Palmer, 1991). This enhancement might have important physiological implications.

Ineffectiveness of L-arginine

In accord with the results of the present study, others have found that L-arginine infusion does not alter blood pressure (Aisaka *et al.* 1989; Girerd, Hirsch, Cooke, Dzau & Creager, 1990) except in very high doses (Nakaki, Hishikawa, Suzuki, Saruta & Kato, 1990) when its effect is not stereospecific and is unlikely to be related to the NO pathway (Calver, Collier & Vallance, 1990). The present study also demonstrated that L-arginine infusion, in a dose which elevated plasma L-arginine levels to approximately 1 mM (Girerd *et al.* 1990), does not alter regional blood flow, cerebrovascular resistance, EEG activity or *ex vivo* cerebrovascular reactivity. Therefore, our data support the view that exogenous L-arginine availability is not a limiting factor in the NO-mediated regulation of cerebral blood flow in the anaesthetized cat.

Conclusion

The present observations clearly indicate that the conductance of the cerebrovascular bed and the resting cerebral blood flow is regulated by L-arginine-derived NO in a regionally heterogenous way and that exogenous L-arginine availability is not a limiting factor in this NO generation. The present results support the proposal that impairment of the endothelial function in various pathophysiological states such as ischaemia and reperfusion (Mayhan *et al.* 1988) or haemorrhagic shock (Kovách, Faragó, Lohinai, Horváth, Fehér, Ottlakán & Szabó, 1991) may predispose to cerebral vasospasm and ischaemia.

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